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Extraction Of Brown Dye From Sesame Seeds

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Abstract: The use of synthetic color in food production has increased in the last decades, since they result in some unsafe product for human consumption. This study reports the extraction of natural brown colourant from sesame seeds using solvent extraction technique. The sesame plant, *Sesamum radiatum*, oil seeds that contain protein, fiber, and healthy fats. The hull gives the seeds a golden-brown hue. The colorant from the sesame seeds were extracted using 1% NaOH solution by centrifugation. The phenolic compound present in the sesame seeds were oxidized and polymerized to form melanin. The extract resembles the melanin pigment and the test were performed to validate the melanin content. The color characteristics were studied using colorimetry at different nanometers. Further the color stability for the extract was evaluated by storing at different environmental conditions (at dark place, at refrigerated, at room temperature and at sun light) for 30 days. The effect of heat and light on the colorant were determined by the transmittance value at interval of 5 days. The study concludes that, the refrigerated colorant has the high colour stability compared with other environmental conditions. This study initiates to use natural colorant in foods, which is currently dominated by synthetic colors.

Index Terms **–Sesamum radiatum, solvent extraction, melanin, natural colorant.**

1.INTRODUCTION

It is widely accepted that "people eat with their eyes first," implying that colour is the most important sensory property in the food and beverage sector. Any dye, pigment, or chemical that imparts colour to food or beverages is referred to as a colour additive [1]. Food colour gives consumer an almost instantaneous perception of a product's freshness, flavor, and quality [12]. This influences whether a consumer chooses to buy that product or something else that appears to be more tempting. Color additives are used in foods to compensate for colour loss caused by light, moisture, and storage conditions, as well as to enhance naturally occurring colors [6].

Because of rising environmental awareness of synthetic colors and their adverse impacts due to their toxicity and non-biodegradable nature, the demand for natural colourants has surged. Natural colourant, on the other hand, is taken from a natural source and may be a better option to synthetic colour [17]. It is environmentally friendly, safe for the body, and its preparation includes the least amount of chemical reactions [9]. Natural colourants do not pose a health risk; in fact, they can occasionally be used as a health-promoting chemical. As a result, the goal of this research is to extract and produce a natural brown colourant that is as stable as possible[12].The current research additionally looks at the characteristics of the colourant that has been extracted.

2. Materials and method

2.1. Chemicals:

1% NaOH solution were prepared using sodium hydroxide pellets [14]. 1g of sodium hydroxide pellets were dissolved in 100 ml of distilled water and dissolved completely. HCl were used to attain the desired pH [8]. Chemicals used were from SRI SHAKTHI FOOD TESTING LABORATORY, COIMBATORE.

2.2. Seed Material:

The black sesame seed were bought from the local store of Indian variety [4].The seeds are manually evaluated; the husk and other foreign materials were removed. Then the seeds are stored in refrigerator until for further use [4].

2.3. Preparation of extracts:

The cleaned sesame seeds were soaked in prepared 1% NaOH solution overnight (approx. 8 hours)[13].Then the mixture is the filtered using Whatman filter paper 1 (11 um) and the filtrate is stored at room temperature. As per the protocol for using sodium hydroxide solution in food product in order to obtain the extract [13]. The mixture is incubated at 60 degree Celsius for 3 hours in water bath and cooled to room temperature. Then the mixture is sealed and refrigerated until the further use [15].

To the obtained extract, 1N HCl is added drop by drop to attain the desired pH 2. Further, the mixture is centrifuged at 1000 rpm for 15 minutes at 25 degree Celsius [3]. The pellets extracted from the centrifuge is washed with triple distilled water twice and colorant is stored in refrigerator for further testing and experiments [6].

2.4.Yield Quantification:

To quantify the yield of colorant from sesame seeds, 1g of seed were extracted with 20 ml of sodium hydroxide solution [4]. The melanin like pigment extracted from the obtained mixture by precipitating the soaked 1% NaOH solution using 1N HCl followed by centrifugation to settle down the precipitate [7].The pellet obtained is then washed twice with triple distilled water and the quantity of the colorant obtained is measured. Thus the colorant obtained from 1g of sesame seed was calculated and expressed in percentage [3].

$$\text{Yield \%} = (\text{weight of obtained extract} / \text{weight of seed taken}) \times 100$$

2.5.UV spectroscopy:

A spectrophotometer (Agilent Cary 300; model SPV IXI) is used here to measure the colour and the colour intensity is evaluated by the means of absorbance values [7]. The ultra-violent visible absorbance spectra of sesame seed extract were examined. Undiluted extract were recorded in various visible region (450nm, 520nm, 680nm) [2]. Synthetic melanin was compared to the spectroscopic properties of extracted melanin-like pigment [2].

2.6.Test for identification of melanin:

According to the literature, the extracted colourant from sesame seeds should be melanin-like pigments. As a result, qualitative tests for the detection of melanin were carried out [8]. Solubility tests for pigments in water, organic solvents (ethanol, acetone), and alkaline reagents are among them (sodium carbonate). Other tests included a reaction with 1% $KMnO_4$ and 3N HCl precipitation [6].

2.7.Stability studies:

The colorant obtained were diluted to 1:5 with distilled water and transmittance values has been noted using colorimeter (Auto Colorimeter AVI 1562) [12]. Distilled water has been taken as reference solution. The transmittance values for the colorant were noted for different wavelengths 450 nm, 520nm and 680 nm [5].

The storage stability of melanin like pigment in 1% NaOH solution were studied by refrigerating the samples at 4 to 7 degree Celsius and at room temperature to evaluate the effect of temperature[9].. And also studied by placing the samples at dark place and at sun light to evaluate the effect of luminescence [5]. The samples were studies for 30 days and the effect of temperature and luminescence were measured using

colorimetry [5]. The transmittance values from the colorimetry is used to calculate the percent degradation in the colour intensity. The colorimetry reading were noted at the interval of every 5 days [16].

3.RESULT AND DISCUSSION:

3.1. Extraction:

The sesame seed extract using one normality NaOH solvents is seen in the Figure 01. The extracts obtained by an extraction process using 1% NaOH as solvent was found to be the darkest in case any other commonly utilized solvents like acetone, acetic acid glacial, acetonitrile, methanol, ethanol, n-propanol, ethanol and hexane [23]. Hence sodium hydroxide can be thought of as good solvents for the extraction of colour pigments from the sesame seeds. Solvent extraction, hydro-distillation, steam distillation, enzyme extraction, and supercritical carbon dioxide extraction are the most commonly used techniques for isolating pigments from plant sources. Alkaline extraction protocol has been followed by which the pigment is isolated and purified [19].

Figure 01: Undiluted extract from sesame seed.

3.2.Yield quantification:

The yield of the colorants obtained from the NaOH extracts of the sesame seed was calculated and expressed in millilitre (Table 01) from known quantities of the seed taken. The quantity of extract had a slight variation in different trials. The overall yield percent of the extract is about 81%. These figures are significantly greater than previously reported melanin yields from other seeds [23], [24]. As a result, the current work provides a low-cost and plentiful supply of melanin, as well as its extraction and characterization.

Trial	Colour	Yield (ml extract/g seed)
Trial -1	Brown	0.84 ml
Trial -2	Brown	0.79 ml
Trial -3	Brown	0.81 ml

Table 01: Yield of the colorants from the oilseeds, obtained in the form of liquid by means of Extraction with NaOH followed by precipitation using HCl and further centrifugation.

3.3.UV spectroscopy:

The absorption spectra of the undiluted extracts of sesame seed within the visible region were recorded so as to study the colour of these extracts. The sesame seed extracts showed an increase in absorbance at lower wavelengths. No distinct peaks were identified for these extracts. The absorption spectra is compared with the synthetic melanin (Figure 02). When compared to synthetic melanin, the UV spectra of melanin extracts from sesame seeds exhibited a considerable match percent. For the identification of melanin pigments from various sources, similar tests are commonly used [11], [22], and [25].

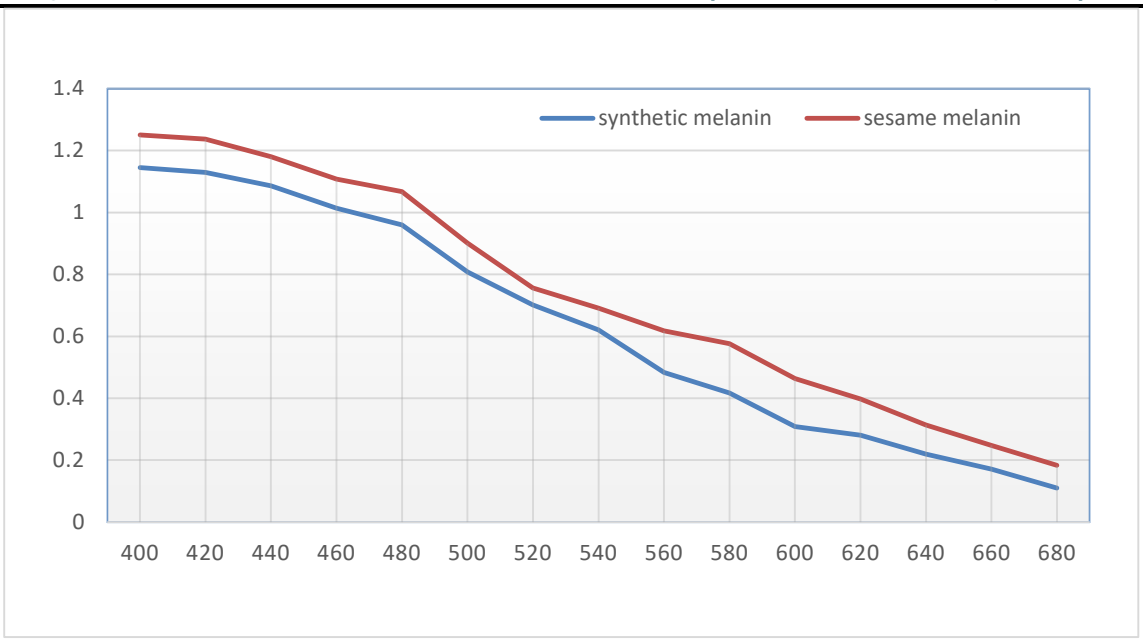

Figure 02: The absorption spectra of the extract from the sesame seed is compared with the synthetic melanin

3.4.Test for melanin pigment:

The extract were water soluble as well as soluble in most organic solvents. They are completely soluble in alkaline solutions, but only partially soluble in strong acids such as nitric, sulfuric, and hydrochloric acids. The outcome of the experiments indicates the presence of quinoid and phenolic groups in melanin. As a result shown below (Table 02), the extracted color is described as a melanin-like pigment. Based on chemical tests frequently used for the identification of melanin, pigments isolated from sesame and mustard seed hulls using alkali followed by acid hydrolysis were recognized as melanin-like pigments [20],[22]..Furthermore, the absorption spectra clearly demonstrating the exponential character of the extracts confirms the pigments' melanin composition, as reported by other researchers [11], [20], and [24].

S.No	Test	Result
1.	Water	Soluble
2.	Ethanol	Decolorized
3.	Acetone	Insoluble
4.	Sodium carbonate	Completely Soluble
5.	3N HCl	Partially soluble
6.	KMnO4	No change

Table 02: Identification parameters for melanin from sesame seed extract

3.5.Stability studies:

The transmittance measurements were used to determine the effect of temperature and light on the extracted melanin pigment. The intensity of the colour steadily decreases as the transmittance value increases. The stability investigation was carried out for 30 days with the interval of 5 days samples has been examined. As a result, we can infer that refrigerated samples have a higher colour retention capacity than other storage modes. The transmittance values at 450nm, 520nm, and 680nm measured using colorimetry and expressed as colour intensity percentage on the influence of light and temperature are shown in the following figures 03, 04 and 05 respectively. The stability of the extracted pigment must be determined in order to assess its suitability for usage as a natural food colourant. The quantitative data on melanin losses when exposed to ambient temperature and light shows that prolonged exposure to these circumstances causes colour loss, which became visible after 15 days of incubation. Similar results regarding the stability of melanin produced from Osmanthus fragrans seeds were reported by [24]. Melanin was determined to be the most stable when refrigerated for one month with minimal colour loss in the current study [20].

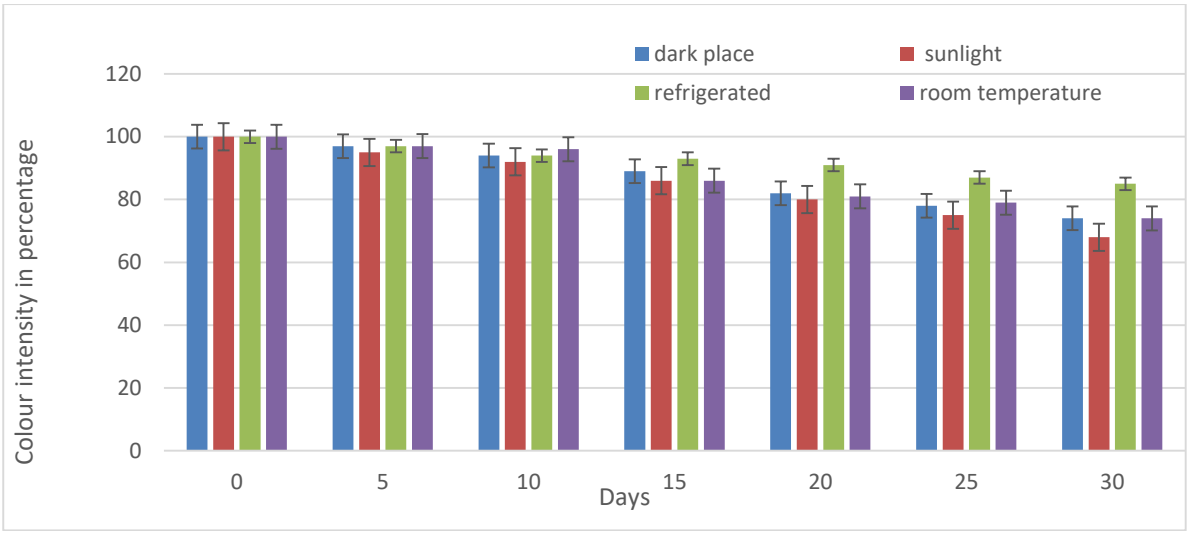

Figure 03: The transmittance value at 450nm measured using colorimetry expressed as color intensity Percentage on the effect of light and temperature.

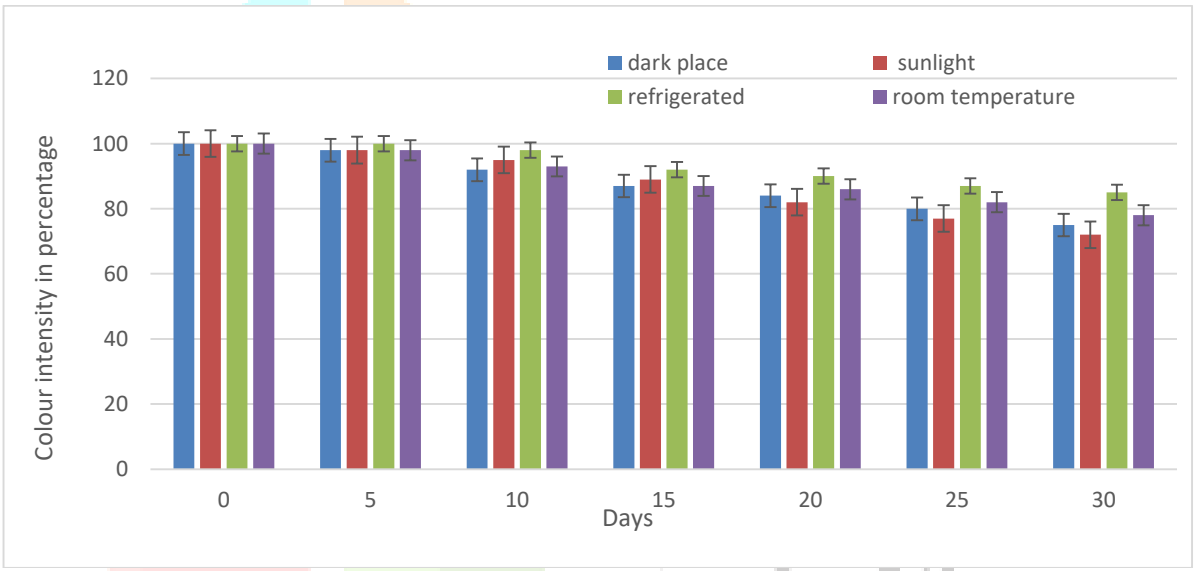

Figure 04: The transmittance value at 520nm measured using colorimetry is expressed as colour intensity Percentage on the effect of light and temperature.

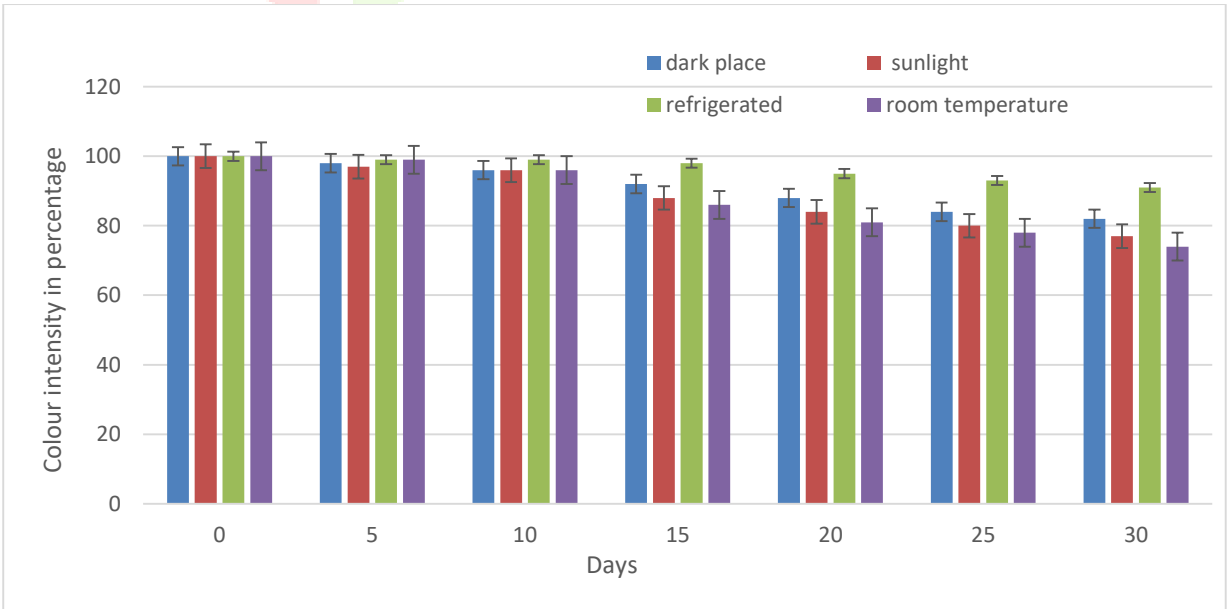

Figure 05: The transmittance value at 680nm measured using colorimetry expressed as colour intensity Percentage on the effect of light and temperature.

Based on the findings, it can be concluded that the NaOH solvent may be used to extract colorant from sesame seeds. The extract's melanin concentration has also been established. The UV-visible spectroscopy of the melanin-like pigment is used to compare the absorption values of the extract to those of synthetic melanin. The extract's storage stability is investigated under various settings, with the conclusion that refrigerator samples are more stable than others. The colorant can also be extracted from the sesame hull and dregs, which could be a good way to repurpose and add value to a by-product.

4. CONCLUSION:

Research on colourant has included all branches of natural colourant has led the way to pure natural food colouring matters. In our research study, the pigment we extracted were identified as melanin like pigment which is desirable to substitute synthetic colourant by pure natural colourant. Development of new Natural food colourant from new source of known pigment, and improvement of existing source. Selection of high yield colourant will mostly led to minor improvements in food industry. Major improvement can be achieved by genetic manipulation of colourant which is not allowed in our country because of naturalness of the colourant would be lost. Extraction of colourant from natural source has a number of advantages, cheaper production, possibly easier extraction, higher yields, no lack of raw material, and no seasonal variations. Natural colourants are already used today like Oleoresin Turmeric, Curcumin, Oleoresin Lutein .It's not unlikely that our new Natural colourant (melanin like pigment from sesame) and it will allowed in near future. A giant leap forward in natural colourant in food industry will be achieved soon.

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